DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
§72-5 (850.1500)

1. **CHEMICAL:** Spiromesifen PC Code No.: 024875

2. **TEST MATERIAL:** BSN 2060 Purity: 96.7%

3. **CITATION:**

Author: Dionne, E.

Title: BSN 2060 - The Full Life-Cycle Toxicity Test with Fathead Minnow (*Pimephales promelas*)

Study Completion Date: March 21, 2002

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MRID No.: 458197-12

DP Barcode: D289385

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Signature: Thomas M Steeger **Date:** 10/25/04

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Pimephales promelas*

Age of Test Organism: <24 hours old (F_0 generation)

Definitive Test Duration: 260 Days (8.5 months)

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

The 8.5-month chronic toxicity of BSN 2060 (spiromesifen) to the full life stage of Fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs (200 embryos/treatment, <24 hours old) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.25, 0.50, 1.0, 2.0, and 4.0 ppb a.i. Mean-measured concentrations were 0.22, 0.44, 0.87, 1.5, and 3.0 ppb a.i.

Following hatching on Day 5, alevins were reduced to 100 per treatment level. On Day 65 (approximately 8 weeks post-hatch), the juveniles were again reduced to 50 per treatment level. On Day 148, six groups of one male and two female per test level were assigned to spawning aquaria, and hatchability trials and early life stage studies were performed for the F_1 generation. Following hatching, the F_1 generation was maintained for 4 weeks. The test was terminated after 260 days.

F_0-generation: No treatment-related effects were observed on any endpoint measured for the F_0 generation, including hatching success, time to hatch, survival through 260 Days, reproductive performance, signs of toxicity, or length or weight.

F_1-generation: A slight statistically-significant reduction in hatching success was observed at the 3.0 ppb a.i. test group compared to the pooled control (74 versus 80%). No treatment-related effects were observed on the time to hatch, signs of toxicity, or on survival or total lengths or weights 4 weeks following hatching.

Based on the hatching success of the F_1 generation, the NOEC is 1.5 ppb a.i., the LOEC is 3.0 ppb a.i., and MATC is 2.1 ppb a.i.

This study is classified as SUPPLEMENTAL. This study does not fulfill the guideline requirements for a fish life-cycle toxicity test because the F_1 generation was only maintained for 4 weeks post-hatch. This study is scientifically valid, and although results do not meet guideline requirements; the information may be useful in a risk assessment.

Results Synopsis:

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)
F_0 Generation		
% Live hatch	3.0	>3.0
Time to hatch	3.0	>3.0
4-week survival	3.0	>3.0
4-week length	3.0	>3.0
8-week survival	3.0	>3.0
8-week length	3.0	>3.0
8-week weight	3.0	>3.0
24-week survival	3.0	>3.0
24-week length (Males)	3.0	>3.0
24-week length (Females)	3.0	>3.0
24-week weight (Males)	3.0	>3.0
24-week weight (Females)	3.0	>3.0
36-week length (Males)	3.0	>3.0
36-week length (Females)	3.0	>3.0
36-week weight (Males)	3.0	>3.0
36-week weight (Females)	3.0	>3.0
# of spawns/female	3.0	>3.0
# of eggs/female	3.0	>3.0

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)
F_1 Generation		
% Live hatch	1.5	3.0
Time to hatch	3.0	>3.0
4-week survival	3.0	>3.0
4-week length	3.0	>3.0
4-week weight	3.0	>3.0
8-week survival	Not determined	Not determined
8-week length	Not determined	Not determined
8-week weight	Not determined	Not determined

NOEC: 1.5 ppb a.i.
LOEC: 3.0 ppb a.i.
MATC: 2.1 ppb a.i.
Endpoint(s) Affected: F_1 % live hatch

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Because the F_1 generation was only maintained for 4 weeks post-hatch (instead of the required 8 weeks), this study does not satisfy guideline requirements for a fish life-cycle toxicity test (§72-5). This study is scientifically valid, and provides supplemental data on the toxicity of BSN 2060 (spiromesifen) to the life cycle of Fathead minnow.

C. Repairability: This study may be upgraded to Core status if data are provided to support that assumptions of no adverse effects on the survival, appearance, or growth of second-generation larvae would have been maintained, had the fish been observed up to 8 weeks post-hatch.

9. GUIDELINE DEVIATIONS:

1. F_1-generation fish were maintained for only 4 weeks, instead of the required 8 weeks.
2. The pH range (6.4-7.9) during the study exceeded recommendations (7.2-7.6).
3. Aeration of exposure solutions was periodically required during periods of the highest biomass loading and feeding.
4. The flow-splitting accuracy was not reported.
5. For at least 3 days, delivery tubes to the 1.0 and 4.0 ppb nominal levels were switched following cleaning; concentrations in the lower level aquaria where the spawning groups had been recently placed were affected. These groups were replaced with adults from the corresponding upper level tank.
6. The high-low ratios exceeded 1.5 for all toxicant levels. Excluding obvious outliers, reviewer-calculated ratios ranged from 2.1-3.8.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of spiromesifen to the full life cycle of Fathead minnows for the purposes of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Prefer Sheepshead minnow (*Cyprinodon variegatus*) or Fathead minnow (*Pimephales promelas*).	Fathead minnow (*Pimephales promelas*)
Source and Acclimation	Embryos were obtained from cultures maintained at the laboratory for 20 years. The culture dilution water was from the same source as dilution water used for testing. There was no mortalities during the 48 hours prior to test initiation.

Guideline Criteria	Reported Information
Age at beginning of test Embryos, 2 to 24 hours old	Embryos, <24 hours old
Feeding Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	Newly hatched fry were fed live brine shrimp nauplii three times daily during the first 30 days post-hatch. After Day 21, a small amount of flaked food was also offered. The juvenile-adult fish were fed twice daily, once with frozen brine shrimp and once with Prostar maintenance flaked food.
Embryo Exposure (4 to 5 Days) Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups. A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used. Parameters measured: · Survival of embryos · Time required to hatch · Hatching success · Survival of fry for 4 weeks Dead and fungused embryos should be counted and removed daily.	Days 0-4 Embryos (< 24 hours old) from six separate spawns were obtained from brood stock and randomly assigned into embryo incubation cups. Each cup contained 50 embryos, with two cups per replicate and two replicate aquaria per treatment level (total of 200 embryos per treatment). Parameters measured: · % live hatch · Time to hatch · Survival of fry/juvenile fish for 4 weeks Mortality was determined daily. Dead embryos were removed.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks) After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: · Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). · Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	Days 5-60 (hatch to approximately 8 weeks) When hatching period was completed, larvae were impartially thinned to 25 per replicate, with four replicates per treatment (100 embryos per treatment), and the larvae were transferred from the incubation cups to the larval growth chambers in the corresponding aquarium. Parameters measured: · Survival of fry/juvenile fish · Total lengths (mm) of all surviving fish at 30 and 60 days (4 and 8 weeks) · Wet weights (g) of all surviving fish at 8 weeks

Guideline Criteria	Reported Information
Juvenile-Adult Exposure (From 8 weeks posthatch to the end of the spawning phase [32-40 weeks]) At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	Days 65 to 260 On Day 65, juvenile fish were reduced to 25 larvae per replicate (50 total fish per treatment). On Day 140, spawning substrates were added to the aquaria. By Day 148, additional aquaria were provided for spawning with one male and two females assigned to each spawning aquaria. There were two spawning aquaria per replicate. The spawning substrates are examined daily and embryos removed, counted, and examined for fertility. Adult exposure was terminated after sufficient spawning had occurred and sufficient numbers of embryo and larval groups had been assessed for survival and growth. Parameters measured: · Survival of adult fish · No. spawns/female · No. eggs/female · No. eggs/spawn · Total lengths (mm) and wet weight (g) of all surviving fish at 176 and 260 days (gender-specific)

Guideline Criteria	Reported Information
Second Generation Embryo Exposure (4 to 5 days) 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	Days 176-260 In each aquarium, 50 embryos from spawns of ≥50 eggs were incubated. Every third spawn was incubated when hatching data was collected for 10 spawns per replicate aquarium. The same test procedures as those employed for the parental generation were used.
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 weeks) After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment). Each group of 2nd generation fish is terminated 8 weeks after hatching. Fish are blotted, weighed, and measured before being discarded.	Days 176-260 Groups of 25 embryos per aquarium were established as the spawning activity permitted (50 embryos for each treatment level). The progeny were from different groups of adult spawners. Each group of F_1-generation fish was terminated 30 days after hatching. Fish were weighed (wet) and measured for total length.

Comments: None.

B. Test System

Guideline Criteria	Reported Information
Test Water Sheepshead Minnow 1. Natural seawater (sterilized and filtered) or a commercial mixture. 2. Natural seawater with a salinity of ≥15 parts per thousand (weekly range of salinity <6% and monthly pH range <0.8 pH units). Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6.	N/A 1. Well water was drawn from a 125-m deep bedrock well, aerated, supplemented with well water supplied by the town of Wareham, MA, and UV-sterilized prior to use. 2. Hardness of 32 to 52 mg/L as $CaCO_3$ and pH of 6.4 to 7.9.
Test Temperature Sheepshead: 30°C. Fathead: 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours.	N/A Range of 24 to 25°C.
Photo-period 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	Photo-period based on graduated photo-period times in Evansville, Indiana. The photo-periods varied from 10 hours and 30 minutes to 15 hours and 45 minutes of light during the study (Table 2, p. 40). The light intensity ranged from 50 to 90 foot-candles.

Guideline Criteria	Reported Information
Dosing Apparatus 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor $\leq$0.5. 3. One control should be used.	1. Intermittent-flow proportional diluter. 2. Five toxicant concentrations with a dilution factor of 0.5. 3. A negative control and a solvent control was used.
Toxicant Mixing 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. A mixing chamber was used for the highest toxicant level (4.0 ppb a.i.). 2. Yes 3. The flow-splitting accuracy was not reported.
Exposure System/Test Vessels Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheepshead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	Glass exposure aquaria (60 x 30 x 30 cm) were used, with a fill volume of 27 L and depth of 15 cm. The larval growth chambers measured 30 x 13 x 25 cm; two larval growth chambers were positioned within each aquarium. During spawning, designated aquaria were divided into three compartments using nylon mesh screen dividers.

Guideline Criteria	Reported Information
Embryo and Fry Chambers 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	The incubation chambers were 7.5 x 16 x 7.5 cm and were positioned at the inflow end of each aquarium. Each incubation chamber held two embryo incubation cups. The embryo incubation cups were 5 cm diameter glass jars with 40 µm mesh Nitex® screen bottoms. A self-starting cap-siphon was used to provide water circulation around the exposed embryos.
Flow Rate Flow rates to adult tanks or larval chambers should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	During the pre-spawning phase, the flow rate was 9.4 volume additions per day. During the spawning phase, the flow rate was 7.8 volume additions per day.
Aeration Dilution water should be aerated to insure dissolved oxygen concentrations at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Dilution water was aerated prior to testing. Aeration of exposure solutions was periodically required during periods of the highest biomass loading and feeding (p. 31).

C. Chemical System

Guideline Criteria	Reported Information
Nominal Concentrations Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate. Toxicant conc. must be measured in one tank at each toxicant level every week.	0 (negative and solvent controls), 0.25, 0.50, 1.0, 2.0, and 4.0 ppb a.i. Toxicant concentrations were measured weekly from alternating replicate aquaria in each test group.

Guideline Criteria	Reported Information
Other Variables 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	1. DO was measured daily in one replicate aquarium. 2. Temperature was measured daily in one replicate aquarium, and was also continuously monitored in one aquarium on both levels of the diluter system. 3. pH was measured daily in one replicate aquarium. Hardness, alkalinity, and specific conductivity were measured weekly during the test in the dilution water control and one treatment level on a rotating basis.
Solvents Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Acetone, 2.2 µL/L until Day 25 and 25 µL/L thereafter. The solvent load was increased following solubility trials, which indicated solubility of the a.i. was maximized at 25 µL/L.

Comments: None.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Data Endpoints must include: · survival of P and F_1 embryos, time required to hatch, and hatching success; · survival and total length of P fish at 4 and 8 weeks after hatching; · weights and lengths of F_1 fish at 8 weeks; · incidence of pathological or histological effects; and · observations of other effects or clinical signs.	Data Endpoints included: · survival of F_0 and F_1 embryos, time required to hatch, and hatching success; · survival and total length of F_0 fish at 4 and 8 weeks after hatching; also wet weights of F_0 fish at 8 weeks after hatching · survival of F_0 fish at 176 Days · total lengths and weights (gender specific) of surviving F_0 fish at 176 and 260 Days · no. eggs/female, no. spawns/female, and no. eggs/spawn of F_0 spawning groups · total lengths and wet weights of F_1 fish at 4 weeks · incidence of pathological or histological effects; · observation of other effects or clinical signs
Raw data included?	Yes

F_0 Results:

Nominal Conc. (ppb a.i.)	Mean Measured Conc. (ppb a.i.) (SD)	% Hatch	4-Week Post-Hatch % Survival[1]	8-Week Post-Hatch % Survival[1]	Day 176 % Survival[2]
Negative Control	<0.025	89	93	93	100
Solvent control	<0.025	87	87	86	98
0.25	0.22 ± 0.04	87	93	92	100
0.50	0.44 ± 0.07	89	92	92	100
1.0	0.87 ± 0.4	89	89	89	100
2.0	1.5 ± 0.3	88	95	95	100
4.0	3.0 ± 0.7	88	95	95	100

Data obtained from Tables 7-9, pp. 49-51.
[1] Relative to 100 larvae/treatment, thinned following hatching completion on Day 5.
[2] Relative to 50 fish/treatment, thinned on Day 65. The test termination (Day 260) survival was not reported.

Mean Total Length (mm)					Wet Weight (g)				
Day 65[1] (8 Weeks)	Day 176[2] (24 Weeks)		Day 260[2] (Test Termination)		Day 65[1] (8 Weeks)	Day 176[2] (24 Weeks)		Day 260[2] (Test Termination)	
	♂	♀	♂	♀		♂	♀	♂	♀
41	74	61	79	63	0.71	4.7	2.5	7.1	2.6
43	74	60	80	61	0.77	5.0	2.4	6.7	2.6
43	73	60	80	64	0.73	4.6	2.3	7.0	3.0
42	75	60	77	64	0.72	4.9	2.4	6.5	3.2
42	75	59	82	61	0.75	4.9	2.3	7.5	2.9
41	73	59	81	63	0.68	4.3	2.1	7.3	2.7
42	74	59	78	66	0.68	4.7	2.3	6.7	3.0

7-10, pp. 49-52.
atment, thinned following hatching completion on Day 5.
ent, thinned on Day 65.

Mean Measured Conc. (ppb a.i.)	Total Number of Spawns[1]	Total Number of Eggs[1]	Number of Eggs/ Spawn	Number of Spawns/Female	Number of Eggs/ Female
Negative Control	24	1574	63	4.0	267
Solvent control	52	5176	98	9.0	908
0.22	51	5891	119	8.6	993
0.44	23	1493	73	3.8	249
0.87	29	1567	59	5.8	322
1.5	65	7047	109	12.0	1300
3.0	47	4755	101	9.4	965

Data derived from Table 11, p. 53.
[1] Values represents mean of two replicates.

Toxicity Observations: No treatment-related effects were observed on any endpoint measured for the F_0 generation, including hatching success, time to hatch, survival through 260 Days, reproductive performance, or length or weight. Hatching was completed by Day 5 for all groups (p. 31).

No treatment-related signs of toxicity were observed (pp. 32-33). Following 176 days of exposure, one fish in the solvent control had lordosis, six fish had deformed opercula (three at the 0.22 ppb a.i. level, and one each at the 0.44, 1.5, and 3.0 ppb a.i. levels), and two fish had damaged caudal fins (one each at the 0.22 and 1.5 ppb a.i. levels). At F_0 termination (Day 260), flared opercula were noted for two fish at the 0.22 ppb a.i. level.

F_1 Results:

Mean Measured Concentration (ppb a.i.)	% Hatch	30-Day Post-Hatch % Survival	30-Day Post-Hatch Length (mm)	30-Day Post-Hatch Wet Weight (g)
Negative Control	79	74	30.8	0.29
Solvent control	81	82	28.5	0.23
0.22	75	78	29.5	0.24
0.44	83	81	29.5	0.25
0.87	77	79	30.0	0.27
1.5	82	81	29.3	0.25
3.0	74*	89	28.4	0.22

Data obtained from Table 12, p. 54

* Significantly reduced compared to the pooled control (Fisher's Exact Test).

Toxicity Observations: A slight statistically-significant reduction in hatching success was observed at the 3.0 ppb a.i. test group compared to the pooled control (74 versus 80%; Table 12, p. 54). No treatment-related effects were observed on the time to hatch, or on survival or total lengths or weights 4 weeks following hatching. Hatching was completed on Day 5 for all groups (Table 13, p. 55).

The only deformity observed in the F_1-generation fish was scoliosis in two larval fish in the 0.44 ppb a.i. level (p. 34).

B. Reported Statistical Results

Data obtained for the F_0 generation that were statistically analyzed included % live hatch; % survival and total length at 4, 8, 24, and 36 weeks post-hatch (Days 35, 65, 176, and 260); total wet weight at 8, 24, and 36 weeks post-hatch; number eggs/female; number spawns/female; and number eggs/spawn (reproductive data collected between Days 148 and 260). The time to hatch data were empirically estimated.

Data obtained for the F_1 generation that were statistically analyzed included % live hatch, time to hatch, and % survival, total length, and wet weight 4 weeks post-hatch.

The negative and solvent control data were compared using a t-Test. If there was a significant difference, the solvent control was used for comparisons (only F_1 length and weight data); otherwise, the controls were pooled for subsequent comparisons. Williams' test was used to

evaluate difference between treatment and the control means ($p \leq 0.05$). The statistical analysis was performed using Fisher's SYSTAT 9 Exact Test (two-tailed) (SYSTAT9®, SPSS, Inc., 1999).

The no observed effect concentration (NOEC) is the highest test concentration causing no adverse effects. The lowest observed effect concentration (LOEC) is the lowest test concentration causing adverse effects. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and the LOEC.

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)
F_0 Generation		
% Live hatch	3.0	>3.0
Time to hatch	3.0	>3.0
4-week survival	3.0	>3.0
4-week length	3.0	>3.0
8-week survival	3.0	>3.0
8-week length	3.0	>3.0
8-week weight	3.0	>3.0
24-week survival	3.0	>3.0
24-week length (Males)	3.0	>3.0
24-week length (Females)	3.0	>3.0
24-week weight (Males)	3.0	>3.0
24-week weight (Females)	3.0	>3.0
36-week length (Males)	3.0	>3.0
36-week length (Females)	3.0	>3.0
36-week weight (Males)	3.0	>3.0
36-week weight (Females)	3.0	>3.0
# of spawns/female	3.0	>3.0
# of eggs/female	3.0	>3.0

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)
F_1 Generation		
% Live hatch	1.5	3.0
Time to hatch	3.0	>3.0
4-week survival	3.0	>3.0
4-week length	3.0	>3.0
4-week weight	3.0	>3.0
8-week survival	Not determined	Not determined
8-week length	Not determined	Not determined
8-week weight	Not determined	Not determined

NOEC: 1.5 ppb a.i.
LOEC: 3.0 ppb a.i.
MATC: 2.1 ppb a.i.
Endpoint(s) Affected: F_1 % live hatch

13. REVIEWER'S STATISTICAL RESULTS:

All adult and offspring endpoints for which reductions from control exceeded 5% were statistically analyzed. For the F_0 generation, data analyzed included wet weight (Day 65), male and female wet weight (Day 176), male wet weight (Day 260, number eggs/spawn, number spawns/female, and number eggs/female. For the F_1 generation, data analyzed included % live hatch, time to hatch (Days 4 and 5), and % survival. With the exception of male wet weight at Day 260, data for all analyzed endpoints satisfied the assumptions of ANOVA (i.e., normal distribution and variance homogeneity), so the NOEC and LOEC for these endpoints were determined using ANOVA. Data for male wet weight at Day 260 were analyzed using the non-parametric Kruskal-Wallis test. For all endpoints, the solvent control was compared to the negative control using a Student's t-test and, upon finding no difference, the two were pooled for comparison to treatment. These analyses were conducted using TOXSTAT statistical software. The NOEC and LOEC for all other endpoints could be visually determined, as reductions from the pooled control did not exceed 4% for any treatment group.

Results synopsis: No statistically-significant differences were observed for any endpoint.

14. REVIEWER'S COMMENTS:

With the exception of hatchling success, for which the study author's analysis detected significant reductions at the highest treatment level, the reviewer's conclusions agreed with the study authors'. The reviewer's analysis detected no significant reductions for any endpoint. Because the study author's conclusions regarding hatchling success are more conservative, they are reported in the Executive Summary and Conclusions sections; the NOEC and LOEC for this study are 1.5 and 3.0 ppb a.i., respectively.

High adult female mortality was observed in the 0.87 ppb a.i. group at F_0 termination (Day 260), so there was insufficient replication to analyze length and weight data for this group (raw data tables on pp. 126 and 128). It was apparent, however, that no treatment-related effects were observed in the other test groups, and the NOEC and LOEC were visually determined. The study author reported that survival was not evaluated at Day 260 because female mortality during the spawning period is common due to the aggressive nature of the male fish (pp. 32-33).

The study author reported that the amount of fungal and microbial growth in the exposure system is directly related to the amount of organic solvent present, and since the reported water solubility of BSN 2060 was 120 ppb, the study was initiated with a relatively low concentration (2.2 µL/L) of acetone. However, since analytical variability was observed between Days 0 and 20, and was believed to be in part to the solubilization rate of BSN 2060 in dilution water in the system, the solvent load was increased on Day 25 to 25 µL/L. This rate was selected based on subsequent solubility trials (result in table on p. 29). Throughout the study, relatively low recoveries (<70% of nominal) were sporadically observed at all test levels, but primarily at the three highest concentrations (Table 4, pp. 42-44). The highest number of occurrences was observed between Days 153 and 202, the period of highest biomass presence. It was reported that considerable effort was expended to keep the exposure aquaria clean. One replicate aquarium, however, continued to have consecutive low recoveries, and the aquarium was replaced, with positive results. It was believed that a fungal and microbial substrate, not removed by the daily cleaning procedure, was affecting the exposure solution concentration. The reviewer-calculated high-low ratios, excluding obvious sporadic outliers, ranged from 2.1-3.8 for all treatment groups, which exceeds the required level of 1.5. Despite the variability, a reasonable effort was made by the laboratory to minimize variation and maximize solubility and demonstrate stability, and with respect to concentration levels, this study is considered a "best effort" by the laboratory. However, since the F_1 generation were not maintained for 8 weeks post-hatch, this study does not fulfill guideline requirements for a fish life-cycle toxicity test (§72-5) and is classified SUPPLEMENTAL.

On Day 160, delivery tubes to the 1.0 and 4.0 ppb nominal levels were inadvertently switched following cleaning; concentrations in only one replicate/level were affected at the 160- and 162-Day sampling intervals. The problem was then identified and corrected. Since the switch

affected only the lower level aquaria where the spawning groups had been recently placed, the spawning groups were replaced with adults from the corresponding upper level tank (p. 30).

A 21-day preliminary experiment was conducted at nominal concentrations of 0 (negative and solvent control), 0.50, 1.0, 2.0, 4.0, and 8.0 ppb to establish dose levels for the definitive study (pp. 27-28). A statistically-significant reduction in embryo hatching success (%) was observed at the 8.0 ppb level compared to the pooled control (87 versus 95%). At study termination (16 days post-hatch), treatment-related reductions in total larval length was observed at the 8.0 ppb level compared to the solvent control, and in wet weight was observed at the 4.0 and 8.0 ppb levels compared to pooled controls. No treatment-related effect on larval survival was observed at any test level.

In order to assess stability, samples of stock solution were analyzed over a period of time equivalent to or exceeding the length of time a stock would be used in the study (p. 26). Samples were collected and analyzed after 0, 4, 6, 12, 18, 25, ad 32 days of storage; recoveries ranged from 95.9 to 103% of nominal (Table 3, p. 41). Results from this study verify that BSN 2060 was stable in the aqueous test system for at least 32 days. During the definitive study, fresh stock solutions were prepared every 2 to 3 weeks (p. 29).

Aeration of exposure solutions was periodically required during periods of the highest biomass loading and feeding (p. 31). The upper level aquaria were aerated from Days 147 to 177, and the lower level aquaria were aerated from Days 157 to 260. To assess the effect of aeration on test concentrations, aerated and unaerated 0.25 (lowest) and 4.0 ppb (highest) exposure solutions were analyzed on Days 157 and 153, respectively. Recoveries were 93% of nominal for the 4.0 ppb aerated solution, compared to 82% of nominal for the 4.0 ppb unaerated solution, and 89% of nominal for the 0.25 ppb aerated solution, compared to 82% of nominal for the 0.25 ppb unaerated solution. These data indicate that aeration had no impact on the ability to maintain exposure levels.

Quality control samples were prepared at each sampling interval and remained with the set of exposure solution samples throughout the analytical process; samples were prepared in freshwater at a nominal concentration range of 0.200-5.00 ppb a.i. (p. 26 and Table 5, pp. 45-47). Recoveries ranged from 85.6-119% (n=111). In addition, there were five outliers outside the acceptable range of 70-120% recovery. A method validation study conducted prior to initiation of the definitive test established a mean recovery of 89.3 ± 6.19% for BSN 2060 from artificial seawater (Table 1A, p. 104). The LOQ was 0.0253 ppb a.i.

This study was performed according to U.S. EPA (FIFRA) Good Laboratory Practice Standards (40 CFR 160, 1993) with the exception of the collection for the water and food contaminant screening analyses. A Quality Assurance Statement was provided.

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16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

wet weight F0 (60 dph)
File: 9712w60 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.011	0.002	0.667
Within (Error)	8	0.025	0.003	
Total	13	0.035		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

wet weight F0 (60 dph)
File: 9712w60 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.738	0.738		
2	0.22	0.735	0.735	0.053	
3	0.44	0.725	0.725	0.264	
4	0.87	0.750	0.750	-0.264	
5	1.5	0.675	0.675	1.318	
6	3.0	0.680	0.680	1.212	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

wet weight F0 (60 dph)
File: 9712w60 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	0.137	18.6	0.003
3	0.44	2	0.137	18.6	0.012
4	0.87	2	0.137	18.6	-0.012
5	1.5	2	0.137	18.6	0.063
6	3.0	2	0.137	18.6	0.057

wet weight F0 (60 dph)
File: 9712w60 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	0.738	0.738	0.738
2	0.22	2	0.735	0.735	0.737
3	0.44	2	0.725	0.725	0.737
4	0.87	2	0.750	0.750	0.737
5	1.5	2	0.675	0.675	0.678
6	3.0	2	0.680	0.680	0.678

wet weight F0 (60 dph)
File: 9712w60 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.738				
0.22	0.737	0.017		1.86	k= 1, v= 8
0.44	0.737	0.017		1.96	k= 2, v= 8
0.87	0.737	0.017		2.00	k= 3, v= 8
1.5	0.678	1.248		2.01	k= 4, v= 8
3.0	0.678	1.248		2.02	k= 5, v= 8

s = 0.055
Note: df used for table values are approximate when v > 20.

male wet weight (176 dpe)
File: 9712mw Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	4.825	4.825	34.500
2	0.22	4.600	4.600	11.000
3	0.44	4.950	4.950	16.500
4	0.87	4.900	4.900	22.000
5	1.5	4.400	4.400	5.500
6	3.0	4.700	4.700	15.500

Calculated H Value = 4.882 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

male wet weight (176 dpe)
File: 9712mw Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

TRANSFORMED	ORIGINAL	GROUP 0 0 0 0 0 0

26

GROUP	IDENTIFICATION	MEAN	MEAN	5 2 6 1 4 3
5	1.5	4.400	4.400	\
2	0.22	4.600	4.600	. \
6	3.0	4.700	4.700	. . \
1	GRPS 1&2 POOLED	4.825	4.825	. . . \
4	0.87	4.900	4.900	. . . . \
3	0.44	4.950	4.950	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

female wet weight
File: 9712fw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.192	0.048	0.511
Within (Error)	7	0.657	0.094	
Total	11	0.849		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

female wet weight
File: 9712fw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	2.475	2.475		
2	0.44	2.350	2.350	0.471	
3	0.87	2.300	2.300	0.659	
4	1.5	2.100	2.100	1.412	
5	3.0	2.350	2.350	0.471	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

female wet weight
File: 9712fw Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.44	2	0.755	30.5	0.125
3	0.87	2	0.755	30.5	0.175

4	1.5	2	0.755	30.5	0.375
5	3.0	2	0.755	30.5	0.125

female wet weight
File: 9712fw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	2.475	2.475	2.475
2	0.44	2	2.350	2.350	2.350
3	0.87	2	2.300	2.300	2.300
4	1.5	2	2.100	2.100	2.225
5	3.0	2	2.350	2.350	2.225

female wet weight
File: 9712fw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	2.475				
0.44	2.350	0.471		1.89	k= 1, v= 7
0.87	2.300	0.659		2.00	k= 2, v= 7
1.5	2.225	0.942		2.04	k= 3, v= 7
3.0	2.225	0.942		2.06	k= 4, v= 7

s = 0.306
Note: df used for table values are approximate when v > 20.

male wet weight 260 dpe
File: 9712mww Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.534	0.307	0.558
Within (Error)	8	4.400	0.550	
Total	13	5.934		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

male wet weight 260 dpe

File: 9712mww Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	6.850	6.850		
2	0.22	7.050	7.050	-0.311	
3	0.44	6.450	6.450	0.623	
4	0.87	7.500	7.500	-1.012	
5	1.5	7.300	7.300	-0.701	
6	3.0	6.700	6.700	0.234	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

male wet weight 260 dpe
File: 9712mww Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	1.861	27.2	-0.200
3	0.44	2	1.861	27.2	0.400
4	0.87	2	1.861	27.2	-0.650
5	1.5	2	1.861	27.2	-0.450
6	3.0	2	1.861	27.2	0.150

male wet weight 260 dpe
File: 9712mww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	6.850	6.850	7.000
2	0.22	2	7.050	7.050	7.000
3	0.44	2	6.450	6.450	7.000
4	0.87	2	7.500	7.500	7.000
5	1.5	2	7.300	7.300	7.000
6	3.0	2	6.700	6.700	6.700

male wet weight 260 dpe
File: 9712mww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

GRPS 1&2 POOLED	7.000			
0.22	7.000	0.234	1.86	k= 1, v= 8
0.44	7.000	0.234	1.96	k= 2, v= 8
0.87	7.000	0.234	2.00	k= 3, v= 8
1.5	7.000	0.234	2.01	k= 4, v= 8
3.0	6.700	0.234	2.02	k= 5, v= 8

s = 0.742
Note: df used for table values are approximate when v > 20.

#eggs per spawn
File: 9712e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	5465.179	1093.036	2.714
Within (Error)	8	3222.250	402.781	
Total	13	8687.429		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

#eggs per spawn
File: 9712e Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	80.250	80.250		
2	0.22	118.500	118.500	-2.201	
3	0.44	73.000	73.000	0.417	
4	0.87	58.500	58.500	1.251	
5	1.5	108.500	108.500	-1.625	
6	3.0	101.000	101.000	-1.194	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

#eggs per spawn
File: 9712e Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	50.352	62.7	-38.250
3	0.44	2	50.352	62.7	7.250
4	0.87	2	50.352	62.7	21.750
5	1.5	2	50.352	62.7	-28.250

6	3.0	2	50.352	62.7	-20.750

#eggs per spawn
File: 9712e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	80.250	80.250	80.250
2	0.22	2	118.500	118.500	83.333
3	0.44	2	73.000	73.000	83.333
4	0.87	2	58.500	58.500	83.333
5	1.5	2	108.500	108.500	104.750
6	3.0	2	101.000	101.000	104.750

#eggs per spawn
File: 9712e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	80.250				
0.22	83.333	0.177		1.86	k= 1, v= 8
0.44	83.333	0.177		1.96	k= 2, v= 8
0.87	83.333	0.177		2.00	k= 3, v= 8
1.5	104.750	1.410		2.01	k= 4, v= 8
3.0	104.750	1.410		2.02	k= 5, v= 8

s = 20.069
Note: df used for table values are approximate when v > 20.

 # spawns per female
File: 9712s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	87.454	17.491	2.038
Within (Error)	8	68.675	8.584	
Total	13	156.129		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

spawns per female

File: 9712s Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	6.500	6.500		
2	0.22	8.550	8.550	-0.808	
3	0.44	3.750	3.750	1.084	
4	0.87	5.800	5.800	0.276	
5	1.5	12.000	12.000	-2.168	
6	3.0	9.350	9.350	-1.123	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

spawns per female
File: 9712s Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	7.351	113.1	-2.050
3	0.44	2	7.351	113.1	2.750
4	0.87	2	7.351	113.1	0.700
5	1.5	2	7.351	113.1	-5.500
6	3.0	2	7.351	113.1	-2.850

spawns per female
File: 9712s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	6.500	6.500	6.220
2	0.22	2	8.550	8.550	6.220
3	0.44	2	3.750	3.750	6.220
4	0.87	2	5.800	5.800	6.220
5	1.5	2	12.000	12.000	10.675
6	3.0	2	9.350	9.350	10.675

spawns per female
File: 9712s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

GRPS 1&2 POOLED	6.220			
0.22	6.220	0.110	1.86	k= 1, v= 8
0.44	6.220	0.110	1.96	k= 2, v= 8
0.87	6.220	0.110	2.00	k= 3, v= 8
1.5	10.675	1.645	2.01	k= 4, v= 8
3.0	10.675	1.645	2.02	k= 5, v= 8

s = 2.930
Note: df used for table values are approximate when v > 20.

number eggs/female
File: 9712ef Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1772383.179	354476.636	3.032
Within (Error)	8	935388.250	116923.531	
Total	13	2707771.429		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

number eggs/female
File: 9712ef Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	587.250	587.250		
2	0.22	992.500	992.500	-1.368	
3	0.44	248.500	248.500	1.144	
4	0.87	322.000	322.000	0.896	
5	1.5	1300.000	1300.000	-2.407	
6	3.0	964.500	964.500	-1.274	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

number eggs/female
File: 9712ef Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	857.887	146.1	-405.250
3	0.44	2	857.887	146.1	338.750
4	0.87	2	857.887	146.1	265.250
5	1.5	2	857.887	146.1	-712.750

6	3.0	2	857.887	146.1	-377.250

number eggs/female
File: 9712ef Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	587.250	587.250	547.500
2	0.22	2	992.500	992.500	547.500
3	0.44	2	248.500	248.500	547.500
4	0.87	2	322.000	322.000	547.500
5	1.5	2	1300.000	1300.000	1132.250
6	3.0	2	964.500	964.500	1132.250

number eggs/female
File: 9712ef Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	547.500				
0.22	547.500	0.134		1.86	k= 1, v= 8
0.44	547.500	0.134		1.96	k= 2, v= 8
0.87	547.500	0.134		2.00	k= 3, v= 8
1.5	1132.250	1.840		2.01	k= 4, v= 8
3.0	1132.250	1.840		2.02	k= 5, v= 8

s = 341.941
Note: df used for table values are approximate when v > 20.

hatching success
File: 9712h Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	208.857	41.771	0.339
Within (Error)	8	986.000	123.250	
Total	13	1194.857		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

hatching success

File: 9712h Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	84.000	84.000		
2	0.22	77.500	77.500	0.676	
3	0.44	83.500	83.500	0.052	
4	0.87	76.500	76.500	0.780	
5	1.5	82.500	82.500	0.156	
6	3.0	74.000	74.000	1.040	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

hatching success
File: 9712h Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	27.853	33.2	6.500
3	0.44	2	27.853	33.2	0.500
4	0.87	2	27.853	33.2	7.500
5	1.5	2	27.853	33.2	1.500
6	3.0	2	27.853	33.2	10.000

hatching success
File: 9712h Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	84.000	84.000	84.000
2	0.22	2	77.500	77.500	80.500
3	0.44	2	83.500	83.500	80.500
4	0.87	2	76.500	76.500	79.500
5	1.5	2	82.500	82.500	79.500
6	3.0	2	74.000	74.000	74.000

hatching success
File: 9712h Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

GRPS 1&2 POOLED	84.000			
0.22	80.500	0.364	1.86	k= 1, v= 8
0.44	80.500	0.364	1.96	k= 2, v= 8
0.87	79.500	0.468	2.00	k= 3, v= 8
1.5	79.500	0.468	2.01	k= 4, v= 8
3.0	74.000	1.040	2.02	k= 5, v= 8

s = 11.102
Note: df used for table values are approximate when v > 20.

survival
File: 9712su Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	222.750	44.550	0.558
Within (Error)	8	638.750	79.844	
Total	13	861.500		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

survival
File: 9712su Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	77.250	77.250		
2	0.22	78.500	78.500	-0.162	
3	0.44	81.000	81.000	-0.485	
4	0.87	78.500	78.500	-0.162	
5	1.5	81.500	81.500	-0.549	
6	3.0	89.500	89.500	-1.583	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

survival
File: 9712su Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	22.418	29.0	-1.250
3	0.44	2	22.418	29.0	-3.750
4	0.87	2	22.418	29.0	-1.250
5	1.5	2	22.418	29.0	-4.250

6	3.0	2	22.418	29.0	-12.250

survival
File: 9712su Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	77.250	77.250	77.250
2	0.22	2	78.500	78.500	78.500
3	0.44	2	81.000	81.000	79.750
4	0.87	2	78.500	78.500	79.750
5	1.5	2	81.500	81.500	81.500
6	3.0	2	89.500	89.500	89.500

survival
File: 9712su Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	77.250				
0.22	78.500	0.162		1.86	k= 1, v= 8
0.44	79.750	0.323		1.96	k= 2, v= 8
0.87	79.750	0.323		2.00	k= 3, v= 8
1.5	81.500	0.549		2.01	k= 4, v= 8
3.0	89.500	1.583		2.02	k= 5, v= 8

s = 8.936
Note: df used for table values are approximate when v > 20.

time to hatch % (day 4)
File: 9712t4 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3499.179	699.836	1.865
Within (Error)	8	3002.250	375.281	
Total	13	6501.429		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

time to hatch % (day 4)

File: 9712t4 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	31.750	31.750		
2	0.22	53.500	53.500	-1.296	
3	0.44	17.500	17.500	0.849	
4	0.87	7.000	7.000	1.475	
5	1.5	18.500	18.500	0.790	
6	3.0	4.000	4.000	1.654	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

time to hatch % (day 4)
File: 9712t4 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	48.602	153.1	-21.750
3	0.44	2	48.602	153.1	14.250
4	0.87	2	48.602	153.1	24.750
5	1.5	2	48.602	153.1	13.250
6	3.0	2	48.602	153.1	27.750

time to hatch % (day 4)
File: 9712t4 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	31.750	31.750	39.000
2	0.22	2	53.500	53.500	39.000
3	0.44	2	17.500	17.500	17.500
4	0.87	2	7.000	7.000	12.750
5	1.5	2	18.500	18.500	12.750
6	3.0	2	4.000	4.000	4.000

time to hatch % (day 4)
File: 9712t4 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

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GRPS 1&2 POOLED	39.000			
0.22	39.000	0.432	1.86	k= 1, v= 8
0.44	17.500	0.849	1.96	k= 2, v= 8
0.87	12.750	1.133	2.00	k= 3, v= 8
1.5	12.750	1.133	2.01	k= 4, v= 8
3.0	4.000	1.654	2.02	k= 5, v= 8

s = 19.372
Note: df used for table values are approximate when v > 20.

time to hatch % (day 5)
File: 9712t5 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3499.179	699.836	1.865
Within (Error)	8	3002.250	375.281	
Total	13	6501.429		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

time to hatch % (day 5)
File: 9712t5 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	68.250	68.250		
2	0.22	46.500	46.500	1.296	
3	0.44	82.500	82.500	-0.849	
4	0.87	93.000	93.000	-1.475	
5	1.5	81.500	81.500	-0.790	
6	3.0	96.000	96.000	-1.654	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

time to hatch % (day 5)
File: 9712t5 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.22	2	48.602	71.2	21.750
3	0.44	2	48.602	71.2	-14.250
4	0.87	2	48.602	71.2	-24.750
5	1.5	2	48.602	71.2	-13.250

6	3.0	2	48.602	71.2	-27.750

time to hatch % (day 5)
File: 9712t5 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	68.250	68.250	61.000
2	0.22	2	46.500	46.500	61.000
3	0.44	2	82.500	82.500	82.500
4	0.87	2	93.000	93.000	87.250
5	1.5	2	81.500	81.500	87.250
6	3.0	2	96.000	96.000	96.000

time to hatch % (day 5)
File: 9712t5 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	61.000				
0.22	61.000	0.432		1.86	k= 1, v= 8
0.44	82.500	0.849		1.96	k= 2, v= 8
0.87	87.250	1.133		2.00	k= 3, v= 8
1.5	87.250	1.133		2.01	k= 4, v= 8
3.0	96.000	1.654		2.02	k= 5, v= 8

s = 19.372
Note: df used for table values are approximate when v > 20.